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Evaluation of the susceptibility of the cultured shrimp *Litopenaeus vannamei* to vibriosis when orally exposed to the insecticide methyl parathion

A. Roque [a,b,*], S. Abad [a], M. Betancourt-Lozano [a], L.M. Garcia de la Parra [a], D. Baird [c], A.L. Guerra-Flores [d], B. Gomez-Gil [a]

[a] *CIAD/Mazatlan, Research Centre for Food and Development/Mazatlan Unit in Aquaculture and Environmental Management, Mexico*

[b] *Instituto de Recerca i Tecnologia Agroalimentaries, Centre d'Acuiculture, Research Institute on Technology, Food and Agriculture, Spain*

[c] *Environmental Group, Institute of Aquaculture University of Stirling, FK9 4LA, UK*

[d] *UAS, Autonomous University of Sinaloa, Mazatlán Sin, Mexico*

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Abstract

The causes of disease in cultured shrimp are difficult to ascertain but there is evidence that disease is correlated with environmental factors. Crustaceans are particularly sensitive to insecticides due to their close phylogenetic relationship with insects. The objective of this study was to investigate whether there was an increased susceptibility of the shrimp *Litopenaeus vannamei* to *Vibrio parahaemolyticus*, when exposed to methyl parathion. The outline of the study was the following: An LC_{50} 96 h was determined to methyl parathion orally offered to juvenile shrimp. Further experiments were carried out in order to determine a concentration that affected the shrimp (verified by measuring the acethylcholinesterase activity) while producing minimal mortalities. This sublethal concentration was used in a susceptibility experiment where methyl parathion was offered to shrimp which were later injected with *V. parahaemolyticus* in a dose expected to kill less than 15%. Probit analysis estimated a 96 h median lethal concentration (LC_{50}) of 1.56 $\mu g\ g^{-1}$. Mortality and AChE activity showed a concentration–response relationship in the exposure treatments. The median inhibitory concentration (IC_{50}) determined was 0.029 $\mu g\ g^{-1}$. These results suggested that a concentration of 0.1 $\mu g\ g^{-1}$ was appropriate for the bacteria–pesticide interaction test as it was able to elicit 11.1% mortality after 10 days of exposure, while producing an AChE inhibition of 57.12%. Cumulative mortalities were significantly increased ($P < 0.01$) in the treatment that combined exposure to methyl parathion and *V. parahaemolyticus* (35.19%) in comparison with methyl parathion or *V. parahaemolyticus* alone (9.26% and 7.41%, respectively).

Keywords: *Litopenaeus vannamei*; Methyl parathion; Acethylcholinesterase; Vibriosis; Pesticide

[*] Corresponding author. Address: Instituto de Recerca i Tecnologia Agroalimentaries, Centre d'Acuiculture, Sant Carles de la Rapita, Spain. Tel.: +34 977 745427/743380; fax: +34 977 744138.
E-mail address: ana.roque@irta.es (A. Roque).

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1. Introduction

Disease in aquatic organisms is thought to arise from an imbalance of the equilibrium which normally exists between host, pathogens and their local environment and is defined as an interruption, cessation or disorder of body functions, systems or organs (Blood and Studdert, 1997). The interaction between environmental stressors, hosts and pathogens is complex, and in a situation where shrimp are being cultured, the establishment of an infection may not only be due to an increase in the number of pathogens but may also be highly dependent on the physiological status of the animals. Under pond conditions, the causes of disease in shrimp are still difficult to ascertain but there is growing evidence that outbreaks of disease are correlated with environmental factors (Overstreet, 1988; Baticados and Tendencia, 1991). However, even if the link between disease and environment can be established, specific data are needed to assess how environmental parameters, such as presence of contaminants, physical damage or water chemistry, influence the onset and development of disease.

Shrimp culture activity has been developed in areas traditionally dedicated to agriculture where pesticides are widely used. Due to increasing environmental regulation, there has been a prevailing tendency to switch from pesticides with persistent action to those which degrade more rapidly such as organophosphates (OPs). However, these new compounds can be extremely toxic and their volumes of application can be considerable (Readman et al., 1992). Nevertheless, even with rapidly-degrading pesticides, non-target organisms can be affected by pulse exposure to these chemicals at non-lethal concentrations (Clark et al., 1993), potentially producing alterations such as reduced growth rate, weakening of the defence mechanisms and pre-disposition to viral and bacterial infections (Mallins and Ostrander, 1991).

Crustaceans are particularly sensitive to insecticides due to their close phylogenetic relationship with insects (Williams and Duke, 1979). In a study by the US Environmental Protection Agency Laboratory, penaeid shrimp were found to be more sensitive to the toxic and ecological effects of most pesticides than fishes and molluscs (Couch, 1978), while early larval stages are generally more sensitive to insecticide toxicity than adults (McKenny, 1986; Juarez and Sanchez, 1989). Methyl parathion is an organophosphorus insecticide used in pest control of agricultural crops (EPA, 1999). In Mexico, methyl parathion is used for controlling pests in alfalfa, cotton, onions, dried beans and other agriculture crops, without any restriction (CICOPLAFEST, 1998). Methyl parathion acts on the nervous system, inhibiting cholinesterase activity (Hassall, 1990). Its presence in the environment can produce undesirable consequences, since it can act on non-target organisms such as crustaceans. As with many other organophosphates, methyl parathion is a highly lipophilic substance (Vale, 1998) and tends to become associated with organic matter when entering aquatic environments (Hertel, 1993).

In aquatic toxicology, most toxicity tests are carried out by means of water-borne exposure. However, for substances that have a tendency to partition out of the water into the solid phase, as it is the case for methyl parathion, dietary or sediment exposure are perhaps the primary routes of uptake. This could be particularly important for benthic organisms such as shrimp which inhabit soft-bottom sediments, although to our knowledge, there are no reports on aquatic toxicity experiments which explicitly expose shrimp via the dietary route.

The objective of this study was to find out whether there would be an increased susceptibility of the shrimp *Litopenaeus vannamei* to *Vibrio parahaemolyticus*, when exposed to methyl parathion via its diet. The inhibition of acethylcholinesterase (AChE) activity in juvenile *L. vannamei* was used as an endpoint to determine the extent of the exposure to methyl parathion. The outline of the study was the following: First an LC_{50} 96 h was determined to methyl parathion orally offered to juveniles of shrimp *L. vannamei*. Based on this information, further toxicity experiments were carried out in order to determine a sufficiently high concentration that affected the shrimp (verified by measuring the acethylcholinesterase activity) while producing minimal mortalities for at least 10 days. This sublethal concentration was subsequently used in a susceptibility experiment where it was offered to shrimp which were later injected with *V. parahaemolyticus* in a dose expected to kill less than 15%. As controls there were a group exposed to only methyl parathion, a group only injected with *V. parahaemolyticus* and group neither exposed to the pesticide nor to the bacterium.

2. Material and methods

2.1. Toxicity tests

2.1.1. Test shrimp

L. vannamei post-larvae 6–7 were obtained from a commercial hatchery in Sinaloa (Mexico). The shrimp were fed 5% of their body wet weight with a commercial diet (39.81% protein, 11.35% lipids, 8.77% humidity and 9.25% ash; Camaronina 30 Purina, Agribrands, Mexico) and were grown in a 100 l raceway re-circulation system (24 °C, 33‰) with constant aeration (Simens, Germany, 1 HP) until they reached the experimental size of 1 g wet weight (2 months of age approximately).